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| (51) International Patent Classification ⁶ : C07H 1/00, C08B 37/06, A01N 43/04 | A1 | (11) International Publication Number: WO 97/34907 (43) International Publication Date: 25 September 1997 (25.09.97) |
| (21) International Application Number: PCT/US97/04205 (22) International Filing Date: 18 March 1997 (18.03.97) (30) Priority Data: 60/013,836 21 March 1996 (21.03.96) US (71)(72) Applicant and Inventor: PLATT, David [US/US]; Building 300, One Kendall Square, Cambridge, MA 02139 (US). (74) Agents: CITKOWSKI, Ronald, W. et al.; Suite 400, 280 North Woodward, Birmingham, MI 48009 (US). | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> | |
| (54) Title: MODIFIED PECTIN MATERIAL (57) Abstract A modified pecting material has a rhamnogalacturan backbone with side chains of neutral sugars dependent therefrom. The first group of side chains comprises relatively short, straight, chains of neutral sugars, and a second group of side chains comprises highly branched chains of neutral sugars. Galactose preferably comprises at least 6 %, by weight, of the neutral sugars, and the molecular weight of the modified pectin material is in the range of 5,000 to 100,000, and most preferably is 10,000. | | |

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MODIFIED PECTIN MATERIAL

Related Application

This patent application claims priority of provisional patent application Serial No. 60/013,836, filed March 21, 1996.

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Field of the Invention

This invention relates generally to carbohydrates. More specifically, the invention relates to pectin derived materials. Most specifically, the invention relates to a particular group of chemically modified pectins which have been found to have therapeutic utility.

10

Background of the Invention

As disclosed in U.S. Patent Application Serial No. 08/024,487 filed March 1, 1993 and entitled "Modified Pectin," the disclosure of which is incorporated herein by reference, certain chemically modified pectins have therapeutic utility in the treatment and prevention of metastatic cancer. These materials are prepared by a general process in which pectin is partially depolymerized by disrupting the rhamnogalacturan backbone thereof, and by breaking the side chains of neutral sugars into smaller units. Typically, the backbone is disrupted under alkaline conditions, and the side chains of neutral sugars are broken up under acidic conditions.

20

In accord with the present invention, particular modified pectins having therapeutic utility and a specific structure have been prepared and identified. The modified pectin material has a shortened rhamnogalacturan backbone with shortened chains of neutral sugars dependent therefrom. In addition, it has been found that in the preferred materials most or all of the methoxyl groups have been

removed from the backbone, and the molecular weight of the modified pectin is approximately 10,000.

Brief Description of the Invention

There is disclosed herein a modified pectin material which comprises a
5 rhamnogalacturan backbone having a repeating sequence of two galacturonic acid
units followed by one rhamnose unit. The modified pectin further includes a first
and second group of side chains of neutral sugars dependent from the backbone via
those rhamnose units which are separated from one another by an intervening
sequence comprising two galacturonic units, a rhamnose unit and two more
10 galacturonic acid units. The first group of side chains comprises straight chains of
neutral sugars and the second comprises branched chains of neutral sugars. The
average molecular weight of the modified pectin is in the range of approximately
5,000 to 100,000, as determined by viscosity measurements. In one preferred
group of materials, the average molecular weight is approximately 10,000.

15 In particular embodiments, the first group of side chains comprises straight
chains of 3-8 linked monosaccharide units, and these units may, in some instances,
be galactose units. In other particular embodiments, the second group of side
chains comprises multiply branched side chains which may be based upon arabinose
units. In certain embodiments, multiply branched side chains of the second group
20 comprises 10-40 linked monosaccharide units.

In a particularly preferred class of materials, the side chains of neutral
sugars comprise, on a weight basis, at least 6% galactose, and most preferably
10% galactose.

In one specific pectin, the second group of side chains comprises a chain of arabinose units having side chains of arabinose units branching therefrom, and the side chains are terminated by either arabinose, galactose, feruloyl or glucose. In yet other instances, the pectin material can include a third group of side chains of neutral sugars comprising a straight chain of 2-4 monosaccharides joined to the backbone through the galacturonic acid units. One particular pectin material comprises, by weight, approximately 28% galacturonic acid, 39% arabinouronic acid and/or arabinose, 12% rhamnose, 10% galactose and 8% glucose.

Brief Description of the Drawing

Figure 1 is a schematic depiction of a generalized molecular structure of the modified pectin material of the present invention.

Detailed Description of the Invention

In accord with the present invention, it has been found that therapeutically useful modified pectin includes a rhamnogalacturan backbone comprising a repeating sequence of two galacturonic acid units followed by one rhamnose unit. Neutral sugar side chains are attached to the backbone through the rhamnose units, and these side chains are attached to those rhamnose units separated by five intervening units, which comprise, in sequence: two galacturonic units, one rhamnose unit and two more galacturonic units. The neutral side chains will include a first group of side chains comprising straight chains of neutral sugars and a second group of side chains comprising branched chains of neutral sugars. In addition, a third group of side chains may also be present, and these will comprise relatively small, straight chains of 2-4 monosaccharides joined to the backbone

through the galacturonic acid units. It has been found that therapeutic utility is best when galactose comprises, by weight, at least 6%, and preferably 10%, of the neutral sugars.

The number and identity of the side chains may vary to some degree, and
5 the materials of the present invention will typically have a molecular weight in the range of 5,000 to 100,000; although it has been found that the modified pectin most preferably has a molecular weight in the range of approximately 5,000 to 50,000, and one specific, preferred material has an average molecular weight of approximately 10,000 as determined by viscosity measurements at 25°C in an
10 Ubbelohde No. 1 viscometer, with sodium-hexamethyl phosphate at 20 mm (pH 4.5) and 0.2% EDTA, 0.9% NaCl. It has also been found that when the reaction conditions (typically acid) employed to break the side chains of neutral sugars into smaller units are varied, the nature of the side chains will vary; but, therapeutic utility remains, provided that the particular rhamnogalacturan backbone structure
15 is maintained, and provided that the side chains include galactose. Therefore, while not wishing to be bound by speculation, Applicant presumes that therapeutic utility is strongly dependent upon the configuration of the backbone, and the presence of galactose in the neutral sugars comprising the side chains.

Referring now to Figure 1, there is shown a schematic depiction of a
20 molecule of modified pectin in accord with the present invention. As depicted, the backbone portion comprises a repeating series of two galacturonic acid units followed by a rhamnose unit. Side chains of neutral sugars branch from rhamnose

units which are separated by at least five intervening units; although as will be noted, not every rhamnose unit which could carry a side chain has one.

As shown in Figure 1, the modified pectin includes straight side chains such as the chain of four galactose units. The pectin also includes highly branched side chains such as the arabinose based, branched side chain. In general, the structure of the highly branched side chain is less variable than is the structure of the relatively straight chain. Figure 1 depicts a typical structure, characteristic of the branched chain. As will be noted, several of the glucose groups are shown as being in brackets; this indicates that these groups are optional. As mentioned previously, other, relatively small, straight, side chains may also be present, and these chains depend from the galacturonic acid units. One such chain is shown in Figure 1 and includes two galactose units. While unmodified pectins generally include a significant percentage of methoxyl groups and the like; in the preferred material of the present invention most, if not all, of such groups have been removed.

A number of specific structures will fall within the definition of the modified pectin of the present invention. One such particular material has been found to include, by weight, approximately 28% galacturonic acid, 39% arabinouronic acid and/or arabinose, 12% rhamnose, 10% galactose and 8% glucose, as determined by gas chromatography/mass spectrometry.

Preparation of the Material

In general, the material of the present invention is prepared by disrupting the rhamnogalacturan backbone of the pectin, and by breaking down the side chains

of neutral sugars therefrom. In this treatment, most of the methoxyl groups are also removed from the backbone. A number of particular procedures may be employed; and in one specific procedure, citrus pectin (70-100 kilodalton 0.5%, from the Sigma Chemical Company of St. Louis, Missouri), which included 10% methoxyl groups, was solubilized and sterilized under UV radiation for 48 hours in distilled water. The total carbohydrate level was determined by the phenol sulfuric acid method. The pH of the solution was increased to 10 with 3N NaOH, maintained for thirty minutes and then decreased to 3.0 with 3N HCl. The solution was maintained at a pH of 3.0 for approximately ten hours and then equilibrated to 6.3. The solution was washed with 70% ethanol and dried with 100% acetone. The material thus produced was analyzed via gas chromatography/mass spectrometry to yield the structural data presented hereinabove.

Other procedures and experimental conditions may be employed to prepare the modified pectin material of the present invention provided that the methodologies depolymerize the backbone and reduce the side chains as detailed above. The material of the present invention has a novel and unique structure characterized by a rhamnogalacturan backbone which is comprised of at least three repeating sequences of two galacturonic acid units followed by one rhamnose unit. In the backbone, these sequences are linearly joined so that the rhamnose unit of one sequence is coupled to the galacturonic acid unit of another. The material further includes two different groups of side chains of neutral sugars dependent from the backbone. The first group of side chains comprise relatively short, straight chains of neutral sugars and the second comprise highly branched chains

of neutral sugars. The side chains are joined to the backbone through the rhamnose units, and are spaced apart from one another by five intervening backbone units, namely an intervening sequence of two galacturonic acid units, one rhamnose unit and two more galacturonic acid units. Most preferably, the neutral sugars of the
5 side chains include at least 6%, and most preferably 10%, by weight galactose. The particular configuration of the material of the present invention has been found to provide a therapeutic material having utility in the treatment of metastatic cancers.

In view of the disclosure and teaching presented herein, various modified
10 pectin materials may be prepared. The foregoing examples and discussion are illustrative of particular embodiments of the present invention, but are not meant to be limitations upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

CLAIMS

1 1. A modified pectin material comprising:
2 a rhamnogalacturan backbone comprising a repeating sequence of two
3 galacturonic acid units followed by one rhamnose unit;
4 said modified pectin further including a first and a second group of side
5 chains of neutral sugars dependent from said backbone, members of said first and
6 second group of side chains being attached to said backbone through rhamnose
7 units thereof which are separated from one another by an intervening sequence
8 comprising two galacturonic acid units, a rhamnose unit and two galacturonic acid
9 units; said first group of side chains comprising straight chains of neutral sugars
10 and said second group of side chains comprising branched chains of neutral sugars;
11 said modified pectin having an average molecular weight in the range of 5,000 to
12 100,000.

1 2. A modified pectin material as in claim 1, wherein members of said
2 first group of side chains are straight chains comprised of 3-8 monosaccharide
3 units.

1 3. A modified pectin material as in claim 2, wherein said
2 monosaccharide units are galactose units.

1 4. A modified pectin material as in claim 1, wherein members of said
2 second group of side chains are multiply branched side chains.

1 5. A modified pectin material as in claim 4, wherein said multiply
2 branched chains are multiply branched chains comprised of arabinose units.

1 6. A modified pectin material as in claim 4, wherein said multiply
2 branched chain comprises 10-40 monosaccharide units.

1 7. A modified pectin material as in claim 1, wherein the members of
2 said second group of side chains comprise a chain of arabinose units having side
3 chains of arabinose units branching therefrom, said side chains being terminated
4 by members selected from the group consisting of: arabinose, galactose, feruloyl
5 groups, glucose, and combinations thereof.

1 8. A modified pectin material as in claim 1, further including a third
2 group of side chains of neutral sugars, members of said third group comprising
3 straight chains of 2-4 monosaccharides which are joined to said backbone through
4 the galacturonic acid units thereof.

1 9. A modified pectin material as in claim 1, wherein the molecular
2 weight of said second side chain is greater than the molecular weight of said
3 backbone.

1 10. A modified pectin as in claim 1, comprising approximately 28%
2 galacturonic acid; 39% arabinouronic acid and/or arabinose; 12% rhamnose; 10%
3 galactose and 8% glucose.

1 11. A modified pectin as in claim 1, wherein the average molecular
2 weight thereof is approximately 10,000.

1 12. A modified pectin as in claim 1, wherein galactose comprises, by
2 weight, at least 6% of the neutral sugars of said side chains.

1 13. A modified pectin as in claim 1, wherein galactose comprises, by
2 weight, 10% of the neutral sugars of said side chains.

1 14. A modified pectin material comprising:
2 a rhamnogalacturan backbone comprised of a repeating sequence of two
3 galacturonic acid units followed by one rhamnose unit;
4 a first side chain, attached to a first rhamnose unit of said backbone, said
5 first side chain comprising a straight chain of monosaccharide units;
6 a second side chain, attached to a second rhamnose unit of said backbone,
7 said first and second rhamnose units being separated by five intervening units of
8 said backbone, said second side chain comprising a multiply branched chain of
9 monosaccharide units; said modified pectin having a molecular weight of
10 approximately 10,000.

1 15. A modified pectin material as in claim 14, wherein said first group
2 of side chains comprise straight chains of galactose units.

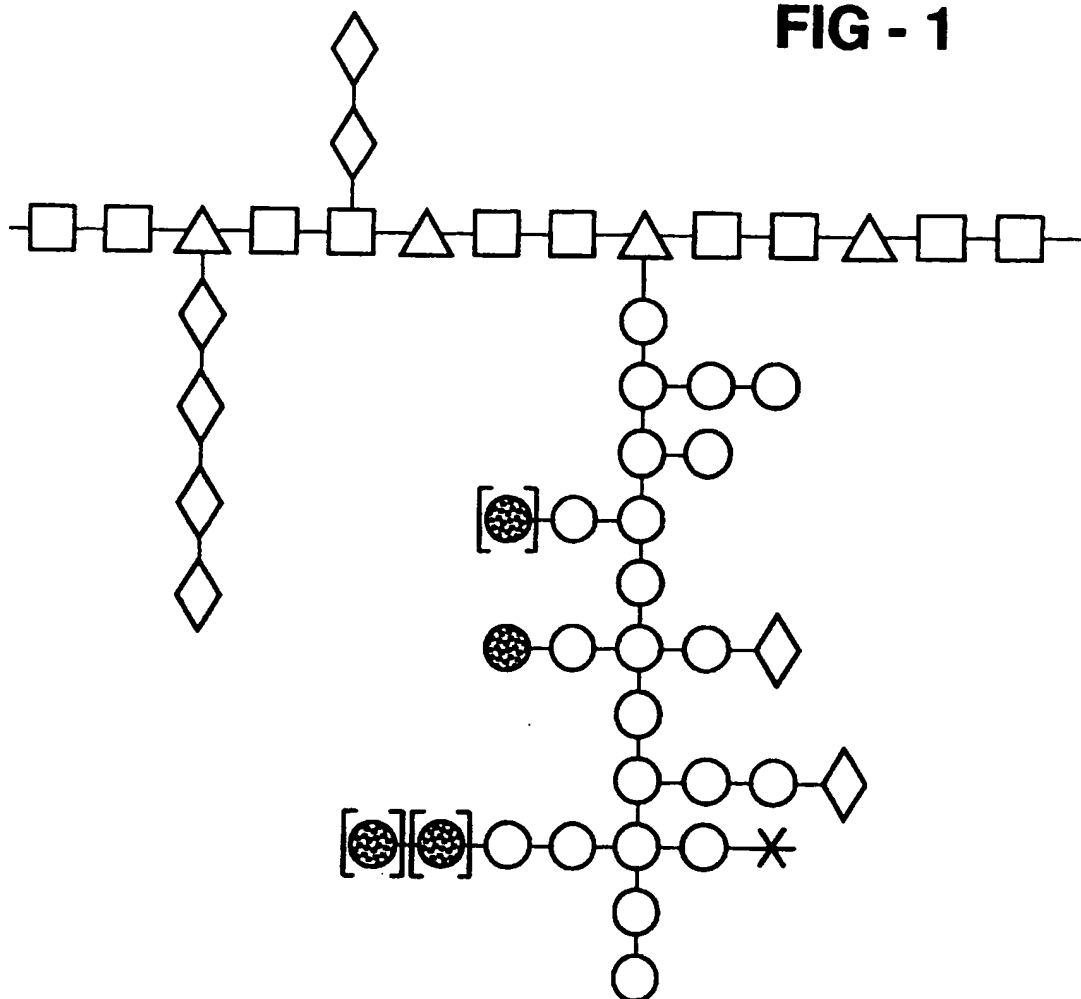
1 16. A modified pectin as in claim 14, wherein members of said second
2 group of side chains comprise a chain of arabinose units having side chains
3 dependent therefrom.

1 17. A modified pectin material as in claim 16, wherein said branching
2 chains comprise chains of arabinose units terminated with a member selected from
3 the group consisting of arabinose, galactose, feruloyl groups, glucose and
4 combinations thereof.

1 18. A modified pectin material as in claim 14, wherein the molecular
2 weight of said second side chain is greater than the molecular weight of said
3 backbone.

1 19. A modified pectin as in claim 14, wherein galactose comprises, by
2 weight, at least 6% of the monosaccharide units constituting said first and second
3 side chains.

- 1 20. A modified pectin as in claim 14, wherein galactose comprises, by
- 2 weight, approximately 10% of the monosaccharide units constituting said first and
- 3 second side chains.

FIG - 1GALACTURONIC
ACID

GALACTOSE



RHAMNOSE



GLUCOSE



ARABINOSE

FERULOYL
GROUP

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04205

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 1/00; C08B 37/06; A01N 43/04

US CL : 536/1.11, 2, 123.1; 514/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/1.11, 2, 123.1; 514/54

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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|-----------|---|-----------------------|
| Y | Chem. abstr., Vol. 108, 1988 (Columbus, OH, USA) page 20, the abstract no. 31316n, FRANZ, G. 'Structure-Activity Relation of Polysaccharides With Antitumor Activity.' Farm. Tijdschr. Belg. 1987, 64(4), pages 301-311, (Eng.), see entire abstract. | 1-20 |
| Y, P | US 5,547,945 A (YE ET AL.) 20 August 1996, abstract. | 1-3, 10 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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